Pattern Recognition by B Cells: The Role of Antigen Repetitiveness Versus Toll-Like Receptors

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Abstract Viruses induce excellent antibody responses due to several intrinsic features. Their repetitive, organised structure is optimal for the activation of the B cell receptor (BCR), leading to an increased humoral response and a decreased dependence on T cell help. Viruses also trigger Toll-like receptors (TLRs), which in addition to increasing overall Ig levels, drive the switch to the IgG2a isotype. This isotype is more efficient in viral and bacterial clearance and will activate complement, which in turn lowers the threshold of BCR activation. Exploiting these characteristics in vaccine design may help us to create vaccines which are as safe as a recombinant vaccine yet still as effective as a virus in inducing B cell responses.

Abbreviations APC: Antigen-presenting cell; BCR: B cell receptor; Ig: Immunoglobulin; IL: Interleukin; IFN: Interferon; LPS: Lipopolysaccharide; RBC: Red blood cell; RNA: Ribonucleic acid; STAT: Signal transducers and activators of transcription; TD: T cell-dependent; TI: T cell-independent; TLR: Toll-like

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receptor; VLP: Virus-like particle; VSV: Vesicular stomatitis virus; VSV-G: Glycoprotein of vesicular stomatitis virus: XID: X-linked immunodeficiency

1 Introduction: Viruses Induce Excellent Antibody Responses

In the eighteenth century, Edward Jenner took advantage of the similarities between cowpox and smallpox to develop the first vaccine to protect against smallpox. Since then, vaccination has been used to induce antibodies against many pathogens and has proven to be an effective mechanism to protect against infectious diseases. Historically, most vaccines are either attenuated or inactivated forms of the infecting pathogens, for example the Sabin or Salk vaccines, respectively (Melnick 1996). While attenuated or inactivated vaccines are usually safe, there remains a small but real risk of reversion to a virulent phenotype. For example, vaccination against poliomyelitis has in effect eradicated the disease from the Western world. In the USA, all reported cases of poliomyelitis since 1982 have resulted from vaccination (MMWR 1986). Given the decreased probability of contracting an infectious disease owing to the success of immunisation programmes, the general public have become less accepting of the risks associated with immunisation. Therefore it has become more important to develop recombinant vaccines which are still able to induce potent and long-lasting neutralising antibody and memory B cell responses to protect against the pathogen, but which are safer and unable to revert back to virulent replicating phenotypes.

The advantage of a virus-based vaccine is that viruses themselves are able to induce strong cytotoxic T and B cell responses, while isolated antigens usually require the nonspecific inflammatory stimuli of an adjuvant to induce a protective immune response (Bachmann et al. 1998). There are a number of features which enable viruses to induce these strong responses. Firstly, viruses present epitopes in a highly structured repetitive array, which allows efficient cross-linking of the BCR. Viruses are also able to activate TLRs and complement, which, in addition to engaging the innate immune system, directly act on B cells. An additional important feature of viruses is their ability to replicate and therefore prolong the exposure to the antigen.

The challenge of modern vaccine design is to use these characteristics of viruses in the design of novel recombinant vaccines to improve their efficacy while retaining the safety of a recombinant vaccine. In this review, we will discuss these characteristics of viruses and explore how they can be used to produce improved vaccines.

2 Epitope Density and the Requirement for T Help

The induction of efficient B cell responses requires two sets of signals: the first is mediated by the BCR, while the second is usually provided by T cells (McHeyzer-Williams and McHeyzer-Williams 2005; Parker 1993). Low doses of antigen or

haptenated proteins only induce B cell responses in the presence of specific T cell help. These antigens are known as T dependent (TD) (Parker 1993). In contrast, repetitive antigens such as haptenated synthetic polymers or polyclonal B cell stimuli such as LPS do not require secondary signals to induce B cell responsiveness. They are T independent (TI) and activate the B cell by extensive cross-linking of the BCR or through the activation of TLRs. Accordingly, TI antigens can be classified into type 1 (TI-1) or type 2 (TI-2) antigens. Generally TI-1 antigens are more potent B cell stimulators and can induce B cells directly with no need for antigen-presenting cells (APCs) or residual T help, while TI-2 antigens require the presence of APCs and some residual T help. TI-1 antigens tend to be polyclonal B cell activators such as LPS; in contrast TI-2 antigens are usually repetitive antigens such as haptenated polymers (Jeurissen et al. 2004; Mond et al. 1995; Vos et al. 2000; see also the chapter by Mond and Kokai-Kun, this volume).

Early studies using flagellin found that the repetitive structure of the polymerised protein is able to induce a TI B cell response, whereas monomeric flagellin or flagellin coupled to RBCs (which lacks the repetitive structure) requires T cell help to induce B cell responses (Feldmann and Basten 1971). It was later shown with haptenated polymers that a minimum number of 12–16 antigenic determinants spaced approximately 10 nm apart are able to activate B cells in the absence of T cell help (Dintzis et al. 1976). This spatially continuous cluster of antigen is coined the immunon and is the minimum immunogenic signal that is required to elicit a TI B cell response. Such responses tend to be TI-2 and do require some residual T cell help. However, highly repetitive antigens which are able to strongly cross-link the BCR are able to stimulate potent IgM responses in the complete absence of T cell help. For example, the glycoprotein of vesicular stomatitis virus (VSV-G) is not a polyclonal B cell activator but can be a TI-1 antigen. VSV has only one neutralising antigenic site, and the neutralising antibodies induced by the different forms of VSV-G all bind to the same epitope (Kelley et al. 1972; Roost et al. 1995). Therefore, it is interesting to study the effects that differences in the structure and organisation of VSV-G have on B cell responses. VSV-G is expressed on the viral envelope in a rigid, quasi-crystaline highly organised way with a spacing of 5–10 nm: the optimal structure for BCR cross-linking and subsequent B cell activation (Dintzis et al. 1976). When VSV-G is expressed on the surface of infected cells (Johnson et al. 1981), or is purified and forms micelles (Petri and Wagner 1979; Simons et al. 1978), it is motile and poorly organised. All these forms of VSV-G will induce high titres of neutralising IgM antibodies without the requirement of T help, indicating that they are TI antigens (Bachmann et al. 1995); however only the highly organised form is a TI-1 antigen. Mice with a deficiency in Bruton tyrosine kinase (BTK) such as X-linked immunodeficiency (XID) mice have immature B cells which can only be activated by TI-1 antigens. In XID mice, only highly organised VSV-G in viral particles, and not poorly organised forms, will elicit a B cell response, indicating that it is a TI-1 antigen when highly organised but not when poorly organised (Bachmann et al. 1995). This is irrespective of whether the virus is able to replicate, because formaldehyde-inactivated VSV is as efficient as WT virus at stimulating B cell responses in XID mice (Bachmann et al. 1995). Since there is only one

neutralising epitope (Kelley et al. 1972; Roost et al. 1995), the differences in the B cell response cannot be attributed to differences in the epitopes recognised and must be attributed to the repetitiveness and organisation of the antigen.

These results corroborated and extended earlier experiments with haptenated beads and bacteria expressing foreign epitopes. In experiments using haptenated synthetic beads, Mond et al. found that highly haptenated beads acted as TI-1 antigens, whereas less densely haptenated beads acted as TI-2 antigens (Mond et al. 1979). Similarly, immunising mice with bacteria engineered to express foreign epitope targeted to either the cell surface (organised antigen) or the periplasmic region (less organised antigen) resulted in TI or TD responses, respectively (Leclerc et al. 1991).

3 Repetitiveness as a Marker for Foreignness

B cell responses against highly organised antigens are either independent from or less dependent on T helper cells than poorly organised or monomeric antigens. This indicates that antigen organisation is an important parameter for B cell activation. Surfaces of viruses, bacteria, and parasites tend to be repetitive, quasi-crystalline in nature. While similarly repetitive self-peptides do exist (for example actin and myosin), they tend to be localised within the cell and not accessible to B cells. In contrast, cell surface molecules almost never form stable clusters such as those found on viral surfaces but remain laterally mobile, rendering them less able to activate B cells. Additionally, membrane-bound antigens are potent inducers of B cell tolerance (Hartley et al. 1991; Russell et al. 1991). This further reduces the probability that membrane-bound antigens will be able to activate self-specific B cells, since such B cells have been deleted from the repertoire.

Together, these observations led to the hypothesis that antigen organisation (i.e. stable, quasi-crystalline surfaces as opposed to repetitive but perhaps mobile membrane proteins) may act as a marker for foreignness. This theory was tested in transgenic mice expressing the membrane form of VSV-G as a self-antigen (Bachmann et al. 1993). When these mice are immunised with poorly organised recombinant VSV-G they are unable to induce a B cell response. However, when highly organised non-replicating VSV particles are used to immunise these mice, the B cell response is restored. The absence of a B cell response cannot be attributed to tolerant T helper cells because depletion of CD4+ cells in normal mice still results in an IgM response to the recombinant VSV-G. This indicates that the IgM response is TI and the inability of disorganised VSV-G to elicit a B cell response is not caused by T cell tolerance but must result from B cell unresponsiveness. Corroborating results were found when tolerant HEL-specific B cells were stimulated in vitro with either soluble HEL or a more organised membrane form of HEL (Cooke et al. 1994). The soluble form of HEL was unable to elicit a B cell response, while a membrane-bound form was able to restore the initial signalling events involved in B cell activation. Collectively these results suggest that B cell unresponsiveness can be overcome by a highly repetitive,

organised, quasi-crystalline antigen. In effect, the immune system is largely unable to distinguish between self and foreign proteins based on antigenic epitopes but does so based on antigenic organisation.

4 Repetitiveness to Break Self-Unresponsiveness: Practical Applications

The ability of highly repetitive antigens to break B cell unresponsiveness has recently been used to generate new types of vaccines for the treatment of chronic diseases. Specifically, by presenting self-peptides in an ordered array self-unresponsiveness can be overcome and autoantibodies can be raised against self-proteins. Virus-like particles (VLPs) have been developed as carriers for the induction of autoantibodies in mice (Chackerian et al. 1999, 2001, 2002; Jegerlehner et al. 2002b; Rohn et al. 2006; Spohn et al. 2005) as well as in humans (Ambuhl et al. 2007). VLPs usually consist of a recombinantly expressed viral coat protein that is assembled into particles. These particles resemble the original virus in terms of having a repetitive structure but carry no genetic information and are therefore unable to infect a host or replicate within it. The self-peptide or self-protein of interest is displayed on the surface of the VLP in a repetitive fashion, leading to a break in B cell unresponsiveness and autoantibody production. As will be discussed in Sect. 6, VLP-based vaccines, which can break B cell unresponsiveness and induce antibodies to neutralise endogenous mediators, such as inflammatory cytokines, are promising candidates for the treatment of a variety of chronic diseases (Dyer et al. 2006).

5 How Does Antigen Repetitiveness Relate to the Magnitude of the Response?

There is a wealth of evidence to suggest that in the absence of adjuvants, soluble and partially aggregated antigens induce poor IgG responses, while highly organised antigens such as bacterial (Feldmann and Basten 1971; Leclerc et al. 1991) and viral (Bachmann et al. 1995; Bachmann et al. 1993; Chackerian et al. 2001; Justewicz et al. 1995) surface proteins are able to induce strong IgG responses. Given that repetitive antigens are usually particulate, whereas poorly organised antigens are often soluble, it remains possible that the observed differences in IgG response are due to these properties rather than a consequence of antigen organisation. For example, particulate antigens are presented to T helper cells in different ways than soluble antigens and B cell responses against proteins in adjuvant are initiated at the interface of the T-B zone (Jacob and Kelsoe 1992; Liu et al. 1991; Van den Eertwegh et al. 1993), whereas particulate antigens initiate a B cell response in the marginal zone or within the B cell follicle (Bachmann et al. 1994; Gatto et al. 2004; Martin et al. 2001).

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